# THE NUTRITIONAL REQUIREMENTS OF TRIBOLIUM CONFUSUM DUVAL

## I. THE SURVIVAL OF ADULT BEETLES ON PATENT FLOUR AND COMPLETE STARVATION DIETS [1]

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### INTRODUCTION

The attention of numerous workers has recently been drawn to the problem of determining the nutritional requirements of insects. The problem has been attacked along two general lines of procedure, namely: (1) by observations on the ability of an organism to survive on a given diet; and (2) by analyses of the different regions of the digestive tract of the organism to determine the presence or absence of the various digestive enzymes. The present series of studies on *Tribolium* concerns the former aspect of the problem. The importance of determining the nutritional requirements of *Tribolium* was emphasized in an earlier paper (Schneider, 1940) on thyroid feeding. There it was stated that " When the fields of the nutritional requirements and the endocrinology of insects have been thoroughly worked, we shall be in a much better position to determine the effect of a given vertebrate hormone extract on a given insect than we are at present."

Chapman (1924), Sweetman and Palmer (1928), Street and Palmer (1935), Nelson and Palmer (1935), Bushnell (1938), and Chiu and McCay (1939), all working with *Tribolium,* studied the nutritional requirements with respect to group biology or population growth and maintenance. Since there are many factors in addition to the nutritional quality of the food medium which affect population growth, i.e., population density, cannibalism, and " conditioning " of the food medium (Park, 1934a), it seemed expedient to study the problem from a more fundamental viewpoint, namely that of determining the individual performances of isolated beetles fed on various diets. From logical considerations it appears that an approach to the problem from this point of

view is likely to lead to a sounder foundation of the nutritional require-
ments of insects *per se,* than can be attained from following any other
line of investigation.

The present investigation has indicated that the starvation control is
of considerable value in nutritional studies, and is particularly desirable
in this case in view of the long life (up to three years) of the organism
on the so-called " normal diet," flour. The term " normal diet " is here
used in the sense that the beetles have been found to grow and reproduce
in it, though it may not really be adequate for optimal physiological
processes. The value of a particular diet can be determined just as
accurately by comparison of the performances of the animals fed on it
with those of animals on complete starvation, as it can by comparison
with those of animals fed the " normal diet," and in this particular case
it is certainly less time-consuming. In other words, if we start with
the starvation diet as the zero point on a scale, all other diets, including
the present so-called " normal diet " can be referred to the zero point
in terms of either positive or negative values, i.e., they will be either
better or worse than no food at all.

## Statement of the Problem

Pearl and Parker (1924) have stated that ". . . when we study
duration of life under normal conditions we are dealing with the com-
bined effects of two variable complexes, inborn organization, on the one
hand, and environment, including renewal of available energy and sub-
stance by food, on the other hand." The former complex, inborn
organization, designated by Ashby (1930) as the " capital " of the or-
ganism, has also been termed the inherent vitality of the organism.
Since the knowledge of the inherent vitality of *Tribolium* is primarily
essential to the knowledge of its general nutritional requirements, the
present investigation has been designed to deal principally with this
phase of duration of life. Hence, the problem may be stated as that of
determining the ability of the adult beetle, *Tribolium confusum* Duval,
to survive under conditions of complete starvation. Several more spe-
cific problems dealing with the influence of various environmental factors
of pre-imaginal life on the ability of the adult beetle to survive conditions
of complete starvation are involved.

## Technique

Park (1934*b*) has described in detail the general technique for han-
dling *Tribolium* cultures in the laboratory, and there is no need for its
repetition here. It is sufficient to state that the beetle spends its entire
life history in flour, and by the use of sieves of various sized mesh, the

adults, eggs, and all immature forms can be separated from the flour for observation. The sex is determined from the pupal characteristics.

Throughout the present investigation, when eggs were used to start an experiment, a sufficient number was taken from a general stock culture, and allowed to hatch in dishes containing a small quantity of patent flour (Ceresota). The date of hatching was determined within 0.5 day, and the larvae were placed in bottles in a definite quantity of flour (indicated in the experiments). The bottles were kept in a darkened constant temperature incubator at a temperature of 29° C., and a relative humidity of approximately 40 per cent. The food was not changed, and the bottles were not sifted until about the twentieth day after hatching, when it became necessary to sift them once a day to collect pupae. The date of pupation was recorded to the nearest 0.5 day (the color of the pupa indicating whether the pupal stage was reached within the past 0.5 or 1.0 day). The pupae were sexed and placed in sterile vials (1.5 cm. diam. × 2.5 cm. tall) and kept in the incubator under the above-mentioned physical environmental conditions. The date of emergence was recorded to the nearest 0.5 day, and the discarded pupa cases were removed from the vials. The vials were closed with corks covered with cellophane, the corks having a filed groove along the side, and the cellophane being perforated so as to maintain a normal oxygen supply within the vials. The experience of the writer in having beetles escape by eating their way through the cork along the filed groove prompted the use of the cellophane covering. This prevented the boring activity in all but a very few vials where the cellophane was broken, and of course these few cases were immediately discarded. The date of death was recorded to the nearest day, and the period from emergence to death (adult survivorship) was calculated.

When only adults of a known age and sex were needed for an experiment, it was necessary to start by collecting pupae from a general or specific stock culture (indicated in the experiments). These were sexed and placed in vials, from which point the procedure was the same as that described above.

In the one experiment where flour-fed controls were used, the vials were supplied frequently with plenty of fresh flour, so that the organism was always presented with an unlimited quantity of food. Data on the weights of pupae, live and dead imagos, have been reserved for a later publication.

The statistical data are based on frequency distributions of the variables studied. The $\frac{\text{Diff.}}{\text{P. E. Diff.}}$ test for significance was used; any value higher than 3 indicating that the difference was probably not due to sampling errors.

## Observations and Results

*Survivorship of Adult Beetles, Taken as Pupae from a General Stock Culture and Subjected to Flour and Starvation Diets upon Emergence*

In the first experiment 432 pupae (216 of each sex) were isolated from a general laboratory stock culture, and were observed daily for emergence. Since these forms were taken from a general stock culture, they were reared under identical, though unknown factors of larval density. Upon emergence, 100 of each sex were submitted to conditions of complete starvation, and 116 of each sex were given a flour diet. Daily observations were made for mortality, and the time of death was recorded. The time intervening between emergence and death was calculated as adult survivorship. Failure of the organism to respond to agitation with a small camel's hair brush was taken as the criterion for death.

Table I presents the data relative to the survivorship of these two groups of organisms.

### Table I

*Survivorship of adult beetles on flour and starvation diets.*

| Group | Range | | Mean number days of life at end of 24 days | Median number days of life at end of 24 days | Standard deviation (days) | Coefficient of variation (per cent) | N |
|---|---|---|---|---|---|---|---|
| | Min. (days) | Max. (days) | | | | | |
| Starved adult males.... | 2.5 | 21.0 | 14.235±0.274 | 14.665±0.343 | 4.062±0.194 | 28.53±1.46 | 100 |
| Flour-fed adult males... | 2.5 | 24.0 | 21.323±0.370 | 24.181±0.464 | 5.908±0.262 | 27.70±1.32 | 116 |
| Starved adult females... | 3.0 | 23.5 | 13.535±0.314 | 14.000±0.394 | 4.648±0.222 | 34.34±1.82 | 100 |
| Flour-fed adult females. | 2.5 | 24.0 | 22.964±0.240 | 24.221±0.301 | 3.826±0.169 | 16.16±0.76 | 116 |
| Total starved adults.... | 2.5 | 23.5 | 13.885±0.213 | 14.593±0.267 | 4.459±0.150 | 32.11±1.19 | 200 |
| Total flour-fed adults... | 2.5 | 24.0 | 22.166±0.220 | 24.202±0.276 | 4.974±0.156 | 22.43±0.74 | 232 |

The data are presented for the sexes separately and then combined. Since all the beetles on starvation were dead at the end of 24 days, it appeared justifiable to compare their survivorship performances with those of the flour-fed group up to the end of 24 days. This procedure obviated the necessity of observing the flour-fed group for their entire life (which may have been up to three years). For a study of the complete survivorship record of flour-fed *Tribolium,* the reader is referred to Pearl, Park and Miner (1941). In the present case it was not possible to calculate mean length of life of the flour-fed group; instead there is calculated the mean number of days of life lived up to the end of 24 days per beetle exposed to risk.

The difference between the mean length of life of the starved beetles (13.885 ± 0.213 days) and the mean number of days of life at the end

## TABLE II

Ungraduated life table for flour-fed and starved adult Tribolium.

| Day of age | Flour-fed | | | | | | | | | | | | Starved | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Male | | | | Female | | | | Both sexes together | | | | Male | | | | Female | | | | Both sexes together | | | |
| | Number living at start of age period | Deaths | Death rate per 1000 | Survivors per 1000 | Number living at start of age period | Deaths | Death rate per 1000 | Survivors per 1000 | Number living at start of age period | Deaths | Death rate per 1000 | Survivors per 1000 | Number living at start of age period | Deaths | Death rate per 1000 | Survivors per 1000 | Number living at start of age period | Deaths | Death rate per 1000 | Survivors per 1000 | Number living at start of age period | Deaths | Death rate per 1000 | Survivors per 1000 |
| 0–1 | 116 | 0 | 0 | 1000 | 116 | 0 | 0 | 1000 | 232 | 0 | 0 | 1000 | 100 | 0 | 0 | 1000 | 100 | 0 | 0 | 1000 | 200 | 0 | 0 | 1000 |
| 1–2 | 116 | 0 | 0 | 1000 | 116 | 0 | 0 | 1000 | 232 | 0 | 0 | 1000 | 100 | 0 | 0 | 1000 | 100 | 1 | 10 | 1000 | 200 | 0 | 0 | 1000 |
| 2–3 | 116 | 3 | 26 | 1000 | 116 | 0 | 0 | 1000 | 232 | 3 | 13 | 1000 | 100 | 3 | 30 | 1000 | 99 | 3 | 30 | 990 | 200 | 3 | 13 | 1000 |
| 3–4 | 113 | 2 | 18 | 974 | 116 | 1 | 9 | 1000 | 229 | 3 | 13 | 987 | 97 | 1 | 11 | 970 | 96 | 3 | 34 | 960 | 196 | 3 | 13 | 987 |
| 4–5 | 111 | 0 | 0 | 957 | 115 | 2 | 17 | 991 | 226 | 2 | 9 | 974 | 96 | 2 | 21 | 960 | 93 | 3 | 31 | 930 | 192 | 3 | 9 | 974 |
| 5–6 | 111 | 0 | 0 | 957 | 113 | 0 | 0 | 974 | 224 | 0 | 0 | 966 | 94 | 1 | 21 | 940 | 88 | 3 | 34 | 880 | 187 | 2 | 9 | 966 |
| 6–7 | 109 | 2 | 18 | 957 | 113 | 0 | 0 | 974 | 224 | 2 | 9 | 966 | 93 | 2 | 22 | 930 | 85 | 3 | 35 | 850 | 181 | 2 | 9 | 957 |
| 7–8 | 105 | 4 | 37 | 940 | 113 | 2 | 18 | 974 | 222 | 4 | 18 | 957 | 91 | 1 | 11 | 910 | 82 | 1 | 12 | 820 | 176 | 4 | 18 | 940 |
| 8–9 | 103 | 2 | 19 | 905 | 113 | 0 | 0 | 956 | 218 | 4 | 19 | 940 | 90 | 4 | 44 | 900 | 81 | 6 | 74 | 810 | 172 | 5 | 29 | 922 |
| 9–10 | 103 | 0 | 0 | 888 | 113 | 0 | 0 | 956 | 214 | 0 | 0 | 922 | 86 | 2 | 23 | 860 | 75 | 5 | 67 | 750 | 167 | 4 | 24 | 922 |
| 10–11 | 103 | 0 | 0 | 888 | 113 | 2 | 18 | 956 | 214 | 0 | 0 | 922 | 84 | 1 | 12 | 840 | 67 | 3 | 43 | 700 | 159 | 5 | 29 | 918 |
| 11–12 | 102 | 1 | 10 | 888 | 111 | 0 | 0 | 956 | 214 | 1 | 5 | 918 | 83 | 2 | 24 | 830 | 57 | 4 | 67 | 670 | 153 | 6 | 38 | 913 |
| 12–13 | 101 | 1 | 10 | 879 | 111 | 0 | 0 | 956 | 213 | 1 | 5 | 913 | 81 | 2 | 24 | 810 | 53 | 8 | 134 | 580 | 148 | 8 | 48 | 909 |
| 13–14 | 99 | 1 | 10 | 871 | 111 | 0 | 0 | 956 | 212 | 1 | 5 | 909 | 75 | 6 | 74 | 750 | 50 | 8 | 138 | 500 | 133 | 8 | 38 | 905 |
| 14–15 | 99 | 0 | 0 | 862 | 111 | 0 | 0 | 956 | 211 | 1 | 5 | 905 | 63 | 12 | 160 | 630 | 42 | 8 | 160 | 420 | 113 | 15 | 101 | 905 |
| 15–16 | 98 | 1 | 10 | 853 | 111 | 0 | 0 | 956 | 210 | 1 | 5 | 905 | 42 | 14 | 333 | 500 | 32 | 10 | 238 | 320 | 84 | 15 | 150 | 901 |
| 16–17 | 96 | 2 | 20 | 853 | 111 | 0 | 0 | 956 | 210 | 0 | 0 | 905 | 32 | 13 | 400 | 420 | 22 | 8 | 303 | 220 | 62 | 23 | 257 | 884 |
| 17–18 | 95 | 1 | 10 | 845 | 109 | 2 | 18 | 939 | 209 | 1 | 5 | 901 | 18 | 5 | 278 | 320 | 17 | 5 | 227 | 170 | 40 | 22 | 262 | 875 |
| 18–19 | 93 | 2 | 21 | 827 | 108 | 1 | 9 | 931 | 205 | 4 | 19 | 884 | 13 | 6 | 462 | 300 | 9 | 4 | 444 | 90 | 30 | 22 | 355 | 862 |
| 19–20 | 93 | 0 | 0 | 819 | 107 | 2 | 19 | 922 | 203 | 2 | 10 | 875 | 7 | 4 | 571 | 180 | 5 | 1 | 200 | 50 | 16 | 14 | 400 | 853 |
| 20–21 | 93 | 0 | 0 | 802 | 105 | 0 | 0 | 905 | 200 | 3 | 15 | 862 | 3 | 3 | 1000 | 130 | 4 | 3 | 750 | 40 | 8 | 8 | 500 | 853 |
| 21–22 | 92 | 1 | 9 | 802 | 105 | 1 | 10 | 905 | 198 | 2 | 10 | 853 | | | | 70 | 1 | 1 | 1000 | 10 | 4 | 4 | 500 | 845 |
| 22–23 | 91 | 1 | 9 | 793 | 104 | 0 | 0 | 905 | 198 | 1 | 5 | 853 | | | | 30 | | | | | 1 | 1 | 1000 | 845 |
| 23–24 | 91 | 0 | 0 | 784 | 104 | 0 | 0 | 905 | 196 | 1 | 5 | 845 | | | | 0 | | | | | | | | 841 |
| 24–25 | | | | | | | | 896 | 195 | | | 841 | | | | | | | | | | | | |

of 24 days of the flour-fed group (22.166 ± 0.220 days) is 8.281 ± 0.306 days (27.06 × P. E.).    The sex difference, though not a significant one in the case of the starved forms, indicates that the males on the average survived slightly longer than the females.    The opposite is true for the flour-fed group, i.e., the females showed a higher mean number of days of life at the end of 24 days than the males.    The latter difference amounts to 1.641 ± 0.441 days (3.72 × P. E.).
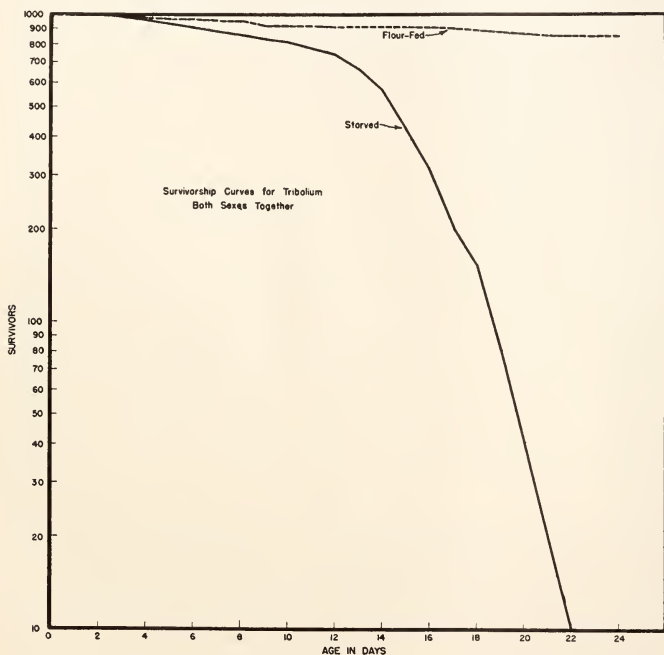


Fig. 1.  Survivorship curves for the flour-fed and starved adults of *Tribolium*, taken as pupae from a general stock culture.  Flour-fed: $N = 232$; observed 24 days.   Starved: $N = 200$; observed entire life.

Table II is the ungraduated life table for these two groups of organisms.    The four columns under each sex heading show: (1) the absolute number of beetles living at the beginning of each day of age and therefore exposed to the risk of dying during that day; (2) the number of deaths actually occurring during each day of age; (3) death

rate for each day of age per 1000 living at the beginning of the day; and (4) survivors per 1000 exposed to risk at the beginning of each day of age.

It is true that a few females of the starved group lived longer than any males of that group, but the fact that a greater number of males survived through the 12- to 14-day period accounts for the higher average survivorship of the males. In the flour-fed group the females took an early lead in survivorship and maintained that lead at an increasing
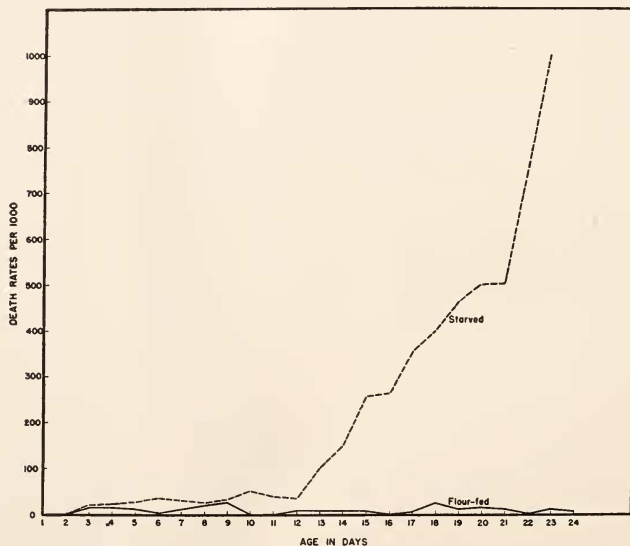


Fig. 2. Observed (ungraduated) death rates (deaths per 1000 exposed to risk) for flour-fed and starved adult *Tribolium,* taken as pupae from a general stock culture.

rate to the end of the 24-day period of observation. Figure 1 shows the survivorship curves for the starved and flour-fed groups, sexes combined.

The only other factor of importance in discussing this particular experiment is the mortality rate. This may be studied from the life table for these two groups of beetles (Table II) and from the mortality curves presented in Fig. 2.

From Fig. 2 it is evident that the mortality rates exhibited no great differences between the flour-fed and starved groups of organisms up to the twelfth day, though during this time the rate for the starved group was slightly higher, on the whole, than that for the flour-fed group. After the twelfth day the mortality rate of the starved beetles went up very rapidly, while that of the flour-fed group continued to fluctuate just a little above the zero line. The point of interest here is that starving the adult beetle for the first 10 or 12 days after emergence does not seriously affect the rate of mortality. If starvation is continued beyond this point, however, the necessity for an exogenous supply of matter and energy for the maintenance of normal physiological processes manifests itself in a sudden rise in the rate of mortality. Whether or not the adult beetle will recover from the effects of starvation if returned to a diet of flour on the twelfth day is a problem for further investigation.

An observation worthy of note here is that the starved beetles produced fecal pellets throughout their entire lives.

The question may be raised as to whether the starved female adults may not have laid eggs and eaten them, and hence may not represent a completely starved group. To answer this point, it can first be stated with certainty that no eggs were ever seen in the vials containing the starved females. This means that the females either ate the eggs immediately after laying them, or that they never laid any eggs at all. Further experimental evidence points to the latter conclusion as the correct one. Virgin females from the flour-fed group were observed carefully for daily egg-laying. In no case were any eggs found under 12 days. At the end of 20 days the cumulative egg-laying record of 185 virgin females in flour was as follows:

49 females had laid 0 eggs at the end of 20 days.
29 females had laid 1 egg at the end of 20 days.
22 females had laid 2 eggs at the end of 20 days.
20 females had laid 3 eggs at the end of 20 days.
21 females had laid 4 eggs at the end of 20 days.
8 females had laid 5 eggs at the end of 20 days.
6 females had laid 6 eggs at the end of 20 days.
13 females had laid 7 eggs at the end of 20 days.
4 females had laid 8 eggs at the end of 20 days.
2 females had laid 9 eggs at the end of 20 days.
3 females had laid 10 eggs at the end of 20 days.
2 females had laid 11 eggs at the end of 20 days.
3 females had laid 12 eggs at the end of 20 days.

It is clear that over 76 per cent of these beetles had laid fewer than 5 eggs each at the end of 20 days.

From this sample of 185 virgin, flour-fed females, it is evident that in the absence of the male, the beginning of egg-laying is decidedly retarded (mated, flour-fed females begin laying 2 or 3 days after emergence). This fact, as well as the low mean length of life of starved females, in addition to the poor body nutrition of the starved group at the time when they would have started laying, all point strongly to the probability that the starved females never laid any eggs during their lives. Furthermore, the fact that the mean length of life of the starved females was slightly less than that of the starved males indicates that even if the females did lay a few eggs and eat them, this action did not contribute greatly to their supply of nutrition.

## TABLE III

*Length of larval period for various larval population densities.*

| Larval density | Range | | Mean length of larval period (days) | Median length of larval period (days) | Standard deviation (days) | Coefficient of variation (per cent) | N |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | Min. (days) | Max. (days) | | | | | |
| (a) 100 larvae per 100 grams flour . . . . . . | 25.5 | 40.0 | $34.095 \pm 0.106$ | $33.431 \pm 0.133$ | $2.719 \pm 0.075$ | $7.97 \pm 0.22$ | 297 |
| (b) 10 larvae per 10 grams flour . . . . . . | 25.5 | 52.0 | $35.922 \pm 0.149$ | $35.281 \pm 0.187$ | $3.726 \pm 0.105$ | $10.37 \pm 0.29$ | 285 |
| (c) 300 larvae per 100 grams flour . . . . . . | 29.5 | 64.0 | $37.460 \pm 0.229$ | $36.038 \pm 0.287$ | $5.642 \pm 0.162$ | $15.06 \pm 0.44$ | 276 |

It is evident that when an experiment such as this is started with pupae from a general stock culture, nothing is known of the population factors under which the larvae developed. If pupae are taken from various stock cultures where larval density factors are almost certain to be unequal, and if larval density does have an influence on adult inherent vitality, then it is clear that the experiment has not been well controlled. In the present experiment, the pupae were all taken from the same stock culture, and hence developed as larvae under identical conditions, though these conditions were probably not optimal. In order to test the influence of larval density on adult inherent vitality, and to find the larval density conducive to the greatest adult vitality, further experiments were made.

### The Influence of Larval Density on Inherent Vitality of Adult Tribolium

*Larval Development.*—For this investigation, eggs were collected from a general laboratory stock culture of *Tribolium,* and allowed to

hatch in a small quantity of flour. Every 12 hours the newly-hatched larvae were collected, and placed in various sized bottles under three different food-density relationships. Each group contained 300 larvae and the density relationships were as follows:

> (*a*) 100 larvae in 100 grams of flour.
> (*b*)  10 larvae in  10 grams of flour.
> (*c*) 300 larvae in 100 grams of flour.

The larvae in (*b*) were kept in 1-ounce bottles; those in (*a*) and (*c*) in half-pint milk bottles. Not only were the survivorship performances of the resulting adults compared for the three series, but also the developmental periods for the larvae.

Table III presents data relative to the length of time spent in the larval stage for the three larval population densities.

From Table III it is evident that the larvae in density (*a*) developed in less time than those from either (*b*) or (*c*). Comparing the mean length of larval period for group (*a*) with that of group (*b*) and (*c*), respectively, we find differences of $1.827 \pm 0.183$ days ($9.98 \times$ P. E.) and $3.365 \pm 0.252$ days ($13.35 \times$ P. E.). Each difference is a statistically significant one, and each points to a more rapid larval development of the (*a*) group. Comparing (*b*) and (*c*), the difference of $1.538 \pm 0.273$ days ($5.63 \times$ P. E.) is also a statistically significant one, and indicates a more rapid rate of development for the (*b*) than for the (*c*) group. From the standpoint of larval development, density (*a*) produced the most rapid rate; density (*b*) the next most rapid rate; and density (*c*) the slowest rate. These observations are substantially in accord with those of Park (1938). The standard deviations indicate that the variation in length of larval period is positively associated with the absolute length of larval period; i.e., with an increase in mean length of larval period comes an increase in the amount of variation. This fact may be grasped more quickly by reference to Fig. 3.

The curve representing density (*a*) is high and narrow; that for density (*c*) is low and broad, tailing off far to the right; while that for density (*b*) falls between these two extremes. Not only is the absolute variation greater for the greater length of larval period, but also the variation relative to the mean (coefficient of variation) as indicated in Table III.

Of the 300 larvae which were started in each series 297 reached pupation in density (*a*), 285 in density (*b*), and 276 in density (*c*), indicating that the larval mortality was influenced by the density factor also. The percentage mortality for the three groups was as follows: (*a*) $1.00 \pm 0.57$ per cent; (*b*) $5.00 \pm 1.26$ per cent; and (*c*) $8.00 \pm$

1.57 per cent. Comparing these percentages we find the following differences: (a) vs. (b), 4.00 ± 1.38 per cent (2.90 × P. E.); (a) vs. (c), 7.00 ± 1.67 per cent; (4.19 × P. E.); and (b) vs. (c), 3.00 ± 2.01 per cent (1.49 × P. E.). Only one of these differences is statistically significant; that existing between groups (a) and (c). Clearly something in addition to chance fluctuation caused the heavier mortality in group (c). In all probability, it was the larval density factor which was responsible for this effect. The difference between (a) and (b) (2.90 × P. E.) is a borderline case of significance, and hence cannot be considered a real difference. The difference between (b) and (c) is
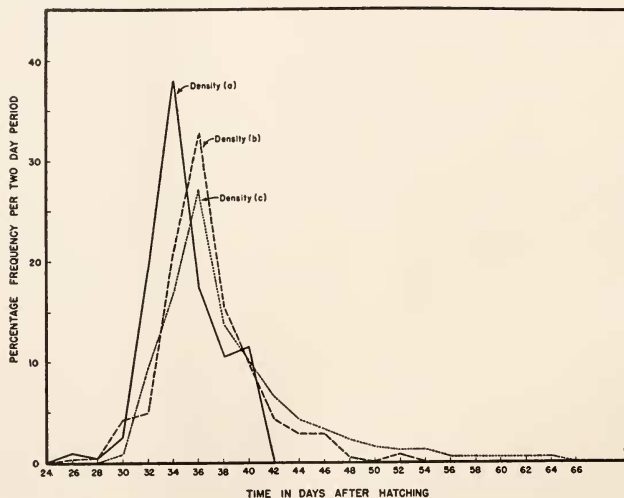


FIG. 3. Frequency distribution of length of larval period for various larval population densities.

clearly not significant. These observations on the density factor as related to larval mortality are also in accord with those of Park (1938).

It is easy to understand the differences that exist between densities (a) and (c), since there are three times as many larvae per unit of environment in (c) as there are in (a). However, the differences between (a) and (b) are more difficult to fathom in view of the fact that the two had the same population density per unit of environment. The only difference lay in the size of the total populations; one was made up of 10 larvae in 10 grams of flour, the other of 100 larvae in 100 grams

of flour. Just why the 100/100 condition was conducive to a lower larval mortality, and more rapid larval development than the 10/10 is not apparent at the moment, though the phenomenon is certainly worthy of further investigation.

*Adult Survivorship.*—After reaching the adult stage, the organisms from the three larval densities described above were isolated and subjected to complete starvation. The survivorship (period from emergence to death) data for the sexes separately, then combined for the three experimental groups are presented in Table IV.

There was only a small and statistically insignificant difference in survivorship between the sexes in densities (*a*) and (*c*). In density

TABLE IV

*Length of life of starved adults, reared under various larval population densities.*

| Larval density | Range | | Mean length of life (days) | Median length of life (days) | Standard deviation (days) | Coefficient of variation (per cent) | N |
|---|---|---|---|---|---|---|---|
| | Min. (days) | Max. (days) | | | | | |
| **Male** | | | | | | | |
| (*a*) 100 larvae per 100 grams flour | 1.5 | 25.0 | 18.739 ±0.118 | 19.353 ±0.148 | 2.086 ±0.083 | 11.13 ±0.45 | 142 |
| (*b*) 10 larvae per 10 grams flour | 3.0 | 23.0 | 16.086 ±0.159 | 16.500 ±0.199 | 2.887 ±0.112 | 17.94 ±0.72 | 150 |
| (*c*) 300 larvae per 100 grams flour | 3.5 | 21.5 | 17.007 ±0.154 | 17.568 ±0.193 | 2.632 ±0.109 | 15.47 ±0.65 | 133 |
| **Female** | | | | | | | |
| (*a*) 100 larvae per 100 grams flour | 3.0 | 24.0 | 18.753 ±0.173 | 19.375 ±0.217 | 3.169 ±0.122 | 16.89 ±0.67 | 152 |
| (*b*) 10 larvae per 10 grams flour | 8.0 | 23.5 | 17.088 ±0.061 | 17.202 ±0.076 | 1.018 ±0.043 | 5.96 ±0.26 | 125 |
| (*c*) 300 larvae per 100 grams flour | 4.0 | 22.0 | 17.547 ±0.169 | 18.333 ±0.212 | 2.918 ±0.119 | 16.62 ±0.70 | 136 |
| **Total** | | | | | | | |
| (*a*) 100 larvae per 100 grams flour | 1.5 | 25.0 | 18.763 ±0.128 | 19.393 ±0.160 | 3.251 ±0.090 | 17.32 ±0.49 | 294 |
| (*b*) 10 larvae per 10 grams flour | 3.0 | 23.5 | 16.414 ±0.105 | 17.046 ±0.131 | 2.593 ±0.074 | 15.79 ±0.46 | 275 |
| (*c*) 300 larvae per 100 grams flour | 3.5 | 22.0 | 17.181 ±0.115 | 18.032 ±0.144 | 2.794 ±0.081 | 16.26 ±0.48 | 269 |

(*b*) this difference is 1.002 ± 0.170 days (5.88 × P. E.). In all three cases, however, the differences indicate that the females survived longer than the males. For the combined sexes, there are large differences in survivorship between the adults reared as larvae, under different population densities. Group (*a*) showed the best survivorship; group (*c*) the next best; and (*b*) the worst. A comparison of the mean length of adult life of group (*a*) with that of group (*b*) and (*c*) respectively, reveals differences of 2.349 ± 0.166 days (14.15 × P. E.) and 1.582 ± 0.172 days (9.20 × P. E.). In each case group (*a*) exhibited a superiority in survivorship which is far outside the limits of chance fluctuation. A comparison of mean survivorship of groups (*b*) and (*c*)

brings to light a difference of $0.767 \pm 0.156$ days ($4.91 \times$ P. E.), which difference is also a statistically significant one. In this case, group ($c$) possessed the greater survivorship value. These differences are illustrated by the survivorship curves in Fig. 4.

The influence of larval density on adult survivorship is clear, but the exact differential effect of the different densities is not so apparent.
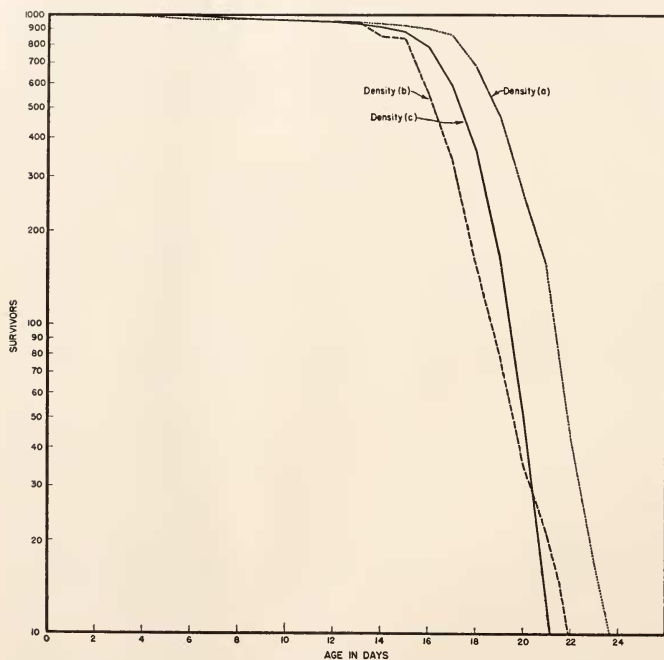


Fig. 4. Survivorship curves of starved adult *Tribolium* reared in various larval population densities.

It is true that group ($a$) which showed the most rapid larval development also exhibited the longest record of survivorship. But group ($b$), which showed the second most rapid larval development, survived the shortest period of time on starvation; and, conversely, group ($c$), which showed the slowest larval development, possessed the second best adult viability. These results indicate the desirability of further work to determine the differential effects on growth, development, and inherent

vitality of various sized total populations kept at a constant density per unit of environment.

This experiment has demonstrated that larval density is a factor which must be controlled in investigations on adult inherent vitality. From this it follows that the " capital " of the newly emerged adult *Tribolium* is not solely dependent upon the inborn organization of the organism, but also upon the environmental influences which have been effective throughout its immature stages. In a study such as that of Greiff (1940), where the length of life of isolated adults of *Drosophila melanogaster* and that of its mutant, Ebony, under conditions of complete starvation were compared, and where it was admitted that ". . . the ebony mutant fly was observed to do better under laboratory conditions than the wild-type fly " (i.e., it produced higher larval densities in the stock bottles from which the flies were isolated), it would obviously have been desirable to control the larval density factor.

### Table V

*Length of larval period for descendants of parents of indicated ages.*

| Age of parents | Range | | Mean length of larval period (days) | Median length of larval period (days) | Standard deviation (days) | Coefficient of variation (per cent) | N |
|---|---|---|---|---|---|---|---|
| | Min. (days) | Max. (days) | | | | | |
| 1 month | 30.5 | 54.0 | $37.516 \pm 0.154$ | $37.416 \pm 0.193$ | $3.830 \pm 0.109$ | $10.21 \pm 0.29$ | 279 |
| 6 months | 25.5 | 48.0 | $33.896 \pm 0.175$ | $33.618 \pm 0.219$ | $4.235 \pm 0.124$ | $12.49 \pm 0.37$ | 265 |

To separate the environmental factor of larval density from the inborn organization variable, and to test the relationship between age of parent and inherent vitality of offspring, further experiments were made.

### Influence of Age of Parents on Vitality of Adult Offspring

*Larval Development.*—Eggs from two different stocks, one a month old, the other six months old, were collected and kept separate for hatching, whereupon 300 larvae from each group were placed in flour in half-pint milk bottles at a density of 100 larvae to 100 grams of food. Every factor in the experimental procedure was held constant except that of the age of the parent generations.

The data on larval development of these two groups of organisms descended from parents of different ages are set out in Table V.

The mean figures for length of larval period for the two groups reveal a difference of $3.620 \pm 0.233$ days ($15.53 \times$ P. E.). The progeny of the six-months-old parents developed at a rate significantly more

rapid than those of parents one month old. The frequency distribution curves in Fig. 5 illustrate this point clearly.
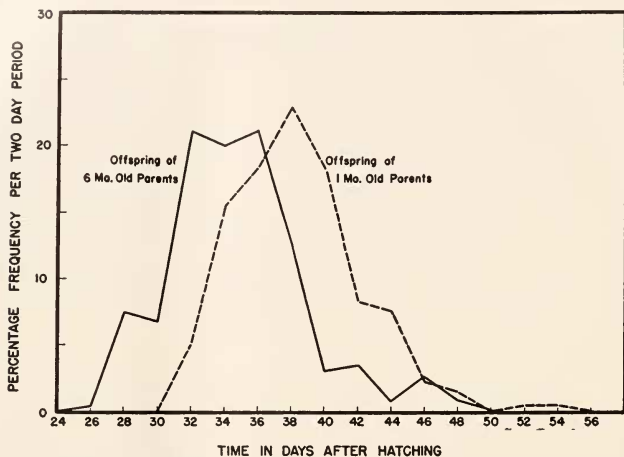


Fig. 5.   Frequency distribution of length of larval period of beetles descended from stocks of various ages.

It is apparent that the six-months-old parents, having reached a more highly productive and more mature period of life, produced progeny which developed more rapidly than those produced by the younger, less productive, one-month-old parents. The variability in length of larval

TABLE VI

*Length of life of starved adults descended from parents of indicated ages.*

| Age of parents | Range | | Mean length of life (days) | Median length of life (days) | Standard deviation (days) | Coefficient of variation (per cent) | N |
|---|---|---|---|---|---|---|---|
| | Min. (days) | Max. (days) | | | | | |
| Male | | | | | | | |
| 1 month | 11.0 | 21.5 | $16.196 \pm 0.136$ | $16.160 \pm 0.170$ | $1.992 \pm 0.096$ | $12.30 \pm 0.60$ | 97 |
| 6 months | 6.0 | 22.5 | $17.450 \pm 0.136$ | $17.791 \pm 0.170$ | $2.129 \pm 0.096$ | $12.20 \pm 0.56$ | 111 |
| Female | | | | | | | |
| 1 month | 4.0 | 26.0 | $16.697 \pm 0.177$ | $17.000 \pm 0.222$ | $2.684 \pm 0.125$ | $16.07 \pm 0.77$ | 104 |
| 6 months | 2.5 | 24.5 | $18.005 \pm 0.174$ | $18.555 \pm 0.218$ | $2.443 \pm 0.123$ | $13.57 \pm 0.69$ | 90 |
| Total | | | | | | | |
| 1 month | 4.0 | 26.0 | $16.455 \pm 0.124$ | $16.716 \pm 0.155$ | $2.618 \pm 0.088$ | $15.91 \pm 0.55$ | 201 |
| 6 months | 2.5 | 24.5 | $17.701 \pm 0.112$ | $18.019 \pm 0.140$ | $2.345 \pm 0.079$ | $13.25 \pm 0.45$ | 201 |

period for the two groups of progeny exhibited no striking difference. The larval mortality for the progeny of one-month- and six-months-old

parents respectively, was $3.67 \pm 1.08$ per cent and $11.67 \pm 1.85$ per cent. The difference here is $8.00 \pm 2.14$ per cent ($3.74 \times$ P. E.) and indicates a significantly greater amount of mortality in the progeny of the six-months-old parents than for those of one-months-old parents.

*Survivorship of Adults.*—Upon emergence, 201 adults from each group were subjected to complete starvation. The numerical data on
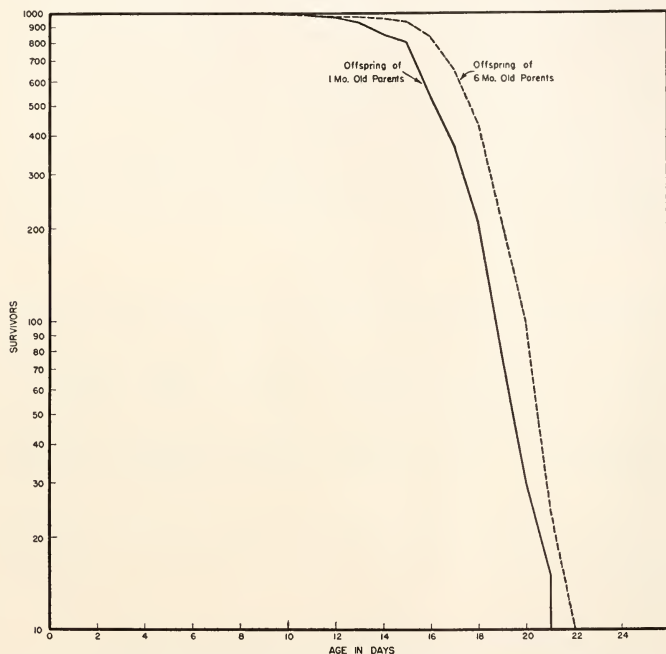


Fig. 6. Survivorship of starved adult beetles descended from parents of indicated ages. $N = 201$ in each group.

length of life of starved adults descended from parents one and six months old respectively are gathered into Table VI.

The sex difference in survivorship of the two groups is not large, though the females again showed a slight superiority. When the survivorship of the two groups is compared for either females, males, or for the combined sexes, there appear large differences. For the females, the difference is $1.408 \pm 0.248$ days ($5.68 \times$ P. E.) ; for the males it is

1.254 ± 0.192 days (6.52 × P. E.); and for the sexes together it is
1.246 ± 0.167 days (7.46 × P. E.). All of these differences are highly
significant and in each case point to a longer survivorship for the progeny
of 6-months-old parents than for those of one-month-old parents. The
difference in survivorship of the two groups (sexes combined) is illus-
trated in Fig. 6.

The sex ratios of the two groups, though not significantly different
from each other, or from a 50–50 ratio, are of interest in view of the
fact that the parents which produced the most rapidly developing larvae
and the most viable adults, also produced the highest proportion of male
offspring. This observation is essentially in accord with that of Law-
rence (1940) on *Drosophila melanogaster*.

Evidently parental age is a factor which must be controlled carefully
in experiments on inherent vitality. It follows that parent age must be
considered in determining the basic foundation of our knowledge of
the nutritional requirements of *Tribolium*.

## TABLE VII

*Survivorship of adult Tribolium subjected, at the indicated ages, to a starvation diet.*

| Starvation begun | Range | | Mean length of life after starvation (days) | Median length of life after starvation (days) | Standard deviation (days) | Coefficient of vari- ation (per cent) | N |
|---|---|---|---|---|---|---|---|
| | Min. (days) | Max. (days) | | | | | |
| (a) At emergence... | 4.0 | 26.0 | 16.455 ±0.124 | 16.716 ±0.155 | 2.618 ±0.088 | 15.91 ±0.55 | 201 |
| (b) At age 20 days . | 2.5 | 26.5 | 16.841 ±0.107 | 17.000 ±0.134 | 3.390 ±0.075 | 20.13 ±0.46 | 456 |
| (c) At age 125 days | 3.0 | 20.0 | 11.676 ±0.174 | 11.687 ±0.218 | 2.892 ±0.123 | 24.76 ±1.39 | 125 |
| (d) At age 220 days | 4.5 | 14.5 | 9.625 ±0.221 | 9.250 ±0.277 | 1.937 ±0.156 | 20.12 ±1.68 | 35 |

In order to determine the influence of the age of the adult beetle on
its ability to survive conditions of complete starvation, a final experi-
ment was performed.

### Influence of Age on Inherent Vitality of Adults

Isolated adults fed a flour diet from the time of emergence to the
time of starvation made up three of the four series of organisms in this
experiment. Starvation was begun at ages 20 days, 125 days, and 220
days respectively in these three groups. Another group representing the
controls were starved from the day of emergence. The beetles in all
four series were descended from parents of equal age (six months) and
were reared under identical larval densities (100 larvae in 100 grams of
flour). The three groups of organisms that were fed for a while and
then starved represent select groups, since only those remaining alive at

the desired time were used. The factor of selection does not impair the value of the experimental data, however, since we are not interested in the viability of those that died before a particular age, but only in the ability of those living at a certain age to survive when transferred to a starvation diet.

The data relative to the survivorship of these four groups of organisms are presented in Table VII.
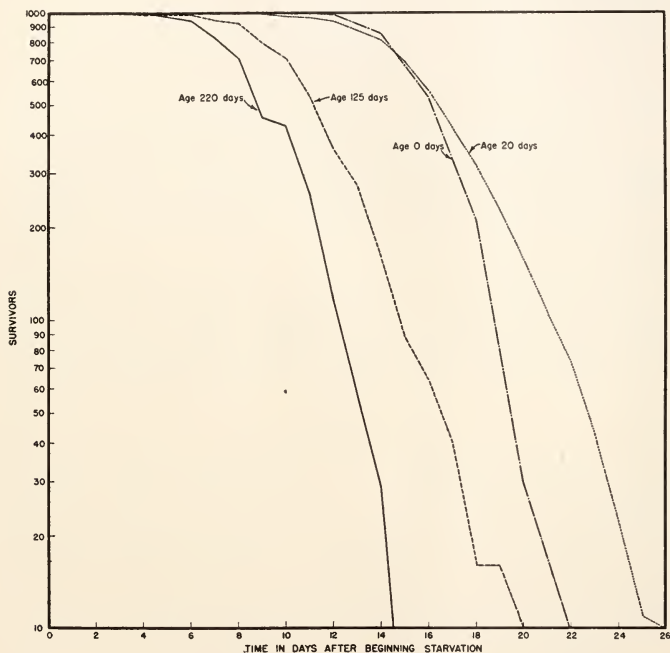


FIG. 7. Survivorship of adult *Tribolium* placed upon a starvation diet at various ages.

From Table VII it is evident that there exist significant differences between each two mean values except in the case of groups (a) and (b), which is only $0.396 \pm 0.164$ days ($2.41 \times$ P. E.). This difference, though statistically not certainly significant, points to a better survivorship of 20-day-old adults than newly emerged adults when both are subjected to starvation. The beetles of the two higher age groups all

survived a shorter period of time on starvation than did those of the two younger groups. The influence of age on the ability of adults to survive under conditions of starvation is illustrated graphically in Fig. 7.

The results suggest that the age at which the beetle survives best under starvation is something more than 0 and less than 100 days. The optimal point is probably in the neighborhood of 20 days. Investigations are now in progress to determine the exact age at which the longest survivorship under starvation occurs.

## SUMMARY

The experimental data set forth in this paper have served to show that:

1. Isolated adults of *Tribolium confusum* Duval, subjected to conditions of complete starvation upon emergence, or shortly thereafter, will survive up to 26.5 days depending upon the conditions of the experiment.

2. Survivorship of starved adults is shortened and the period of larval development is lengthened by an increase in larval population density.

3. Survivorship of starved adults is significantly longer and the period of larval development is significantly shorter for progeny of 6-months-old parents than for those of one-month-old parents.

4. Survivorship of starved adults is shortened with increasing age.

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